Remarks

Claims 1 to 5, 7 to 20 and 22 to 39 are pending in the present case of which claims 1, 12 and 32 are in independent form.

Claims 1 to 5, 7 to 9, 11 to 12, 19 to 20, 22 to 24, 26 to 33, and 36 to 37 were rejected under 35 USC 103(a) as being unpatenable over Uhlmann et al. (Electrophoresis 1999, hereinafter referred to as "Uhlmann '99") in view of U.S. Patent 6,258,568 to Nyren at al. (hereinafter referred to as "Nyren').

Claims 12 to 16, 18 and 38 were rejected under 35 USC 103(a) as being unpatenable over Uhlmann '99 in view of U.S. Patent 6,258,568 to Nyren at al. and in further view of U.S. Patent 5,786,146 to Herman.

Claim 17 was rejected under 35 USC 103(a) as being unpatenable over Uhlmann '99 in view of U.S. Patent 6,258,568 to Nyren at al. and in further view of U.S. Patent 5,786,146 to Herman as applied to claims 12 and 38 and further in view of U.S. Patent Pub. No. US 2003/0232351 TO fEINBERG.

Claims 10, 25, 34 and 39 were rejected under 35 USC 103(a) as being unpatenable over Uhlmann '99 in view of U.S. Patent 6,258,568 to Nyren at al. as applied to claims 1 and 12, and in further view of U.S. Patent 7078168 to Sylvan.

Claim 35 was rejected under 35 USC 103(a) as being unpatenable over Uhlmann '99 in view of U.S. Patent 6,258,568 to Nyren at al. and in further view of U.S. Patent US2002/0086324 to Laird.

Claim 37 was rejected under 35 USC 103(a) as being unpatenable over Uhlmann '99 in view of U.S. Patent 6,258,568 to Nyren at al. as applied to claims 1 and 8, and in further view of U.S. Patent 5,602,000 to Hyman.

Uhlmann '99 discloses a method for identifying changes in methylation patterns by two dimensional (2 D) DNA fingerprinting. The study further supports that changes in methylation status of a DNA molecule indicate the presence of a tumor.

A restriction enzyme digested DNA sample, was, subsequent to being immobilized in agarose beads, subjected to bisulphite treatment. After termination of the reaction and washing of the beads the so treated DNA of interest, still contained in the

beads, was amplified by PCR. Separate PCR reactions of sense and antisense strands of the DNA were performed (see Fig. 1).

The respective amplification products were gel extracted and cloned using a Topo TA cloning Kit (Invitrogen, NL) to produce single stranded DNA and were then sequenced by the dideoxynucleotide chain-termination method. (see 2.5 p. 1750, right column to 1751, right col., l. 3 as well as Fig.1)

Using this method the methylation state of the cloned spot DNA fragment was determined. In particular and as shown in Fig. 4 of the paper, methylated cytosine, which did not change during the bisulfite treatment, continued to appear as cytosine (C), while unmethylated cytosine, which was converted to uracil during the bisulfite treatment, appeared in the amplification product as thymine (T). The data presented in Fig. 4 supports that differential methylation indeed indicates tumor presence.

Uhlmann '99 employed 2-D DNA finderprinting in view of its improved detection capability compared to one-dimensional fingerprinting, in particular its improved capability to detect somatic changes in genomic DNA (see abstract and Introduction). Uhlmann '99 concluded that 2-D DNA finderprinting can serve as a useful tool in genome-wide screenings for methylation differences between constitutional and tumor DNAs.

Uhlmann '99, relies, as other references, e.g., Eads et al. (Nuc. Acid Res,, Vol. 8(8): e32i-vii (2000)) and US. Patent 6,251,594, Gonzalgo et al (2001) as discussed in detail in previous Office Actions as well as Laird (see above), on bisulfite treatment of DNA (see also background section of the present invention).

Nyren discloses a method for sequencing DNA based on the detection of bases incorporation by the release of pyrophosphate (PP_i) and simultaneous enzymatic nucleotide degradation, especially of non-incorporated nucleotides. The nucleotide degradation is said to avoid washing to remove non-incorporated nucleotides, also allowing the polymerase to be recycled. The method is said to permit the sequencing reaction to be continuously monitored in real time.

In ascertaining the differences between the claimed invention and the prior art, the Office conceded that Uhlmann '99 does not teach a method wherein the amplification primer has a label that forms an anchor for removal of single stranded amplified nucleic acid molecules.

The Office also acknowledged that Uhlmann '99 does not teach that the amplified nucleic acids were sequenced using a real-time sequencing method.

However, the Office expressed the opinion that these teachings are provided by Nyren.

In particular, the Office expressed the opinion that Nyren teaches an alternative sequencing method, in which PCR is performed using one or more PCR primers that carry a functional group such as a biotin which permits subsequent immobilization (col. 8, lines 22 to 31) and that real-time sequencing provides a wide variety of desirable advantages.

The Office concluded that it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Uhlmann '99 by using pyrosequencing as taught by Nyren to determine the sequence of the amplified DNA fragment.

Uhlmann '99's amplification primers are not detectably labeled. Uhlmann '99's amplification product is cloned to produce single stranded DNA. This cloning follows Unhmann '99's amplification and precedes the sequencing. ("[P]lasmid DNA of positive clones . . . were sequenced by the dideoxynucleotide chain-termination method." (see 2.5, pages 1751 and 1752)).

Claim 1 recites:

(b) amplifying said nucleic acid molecule . . . via at least one amplification primer . . . detectably labeled with a detectable label that forms an anchor for removal of single stranded amplified nucleic acid molecules to generate a single stranded amplified nucleic acid. . . *[emphasis added]*

Claims 12 and 32 contain similar language.

Applicants note again that the person skilled in the art would be reluctant to make the modification to Uhlmann that the Office suggested, namely detectably label Uhlmann '99's amplification primers as it would interfere with Uhlmann '99' s subsequent cloning step. For example, U.S. Patent 6,589,736 to Rothschild et al. discloses in its background section, "PCR products that are biotinylated are not suitable material for cloning." (col. 7, starting on line 23). The same patent states also in col. 34, starting on line 40 that "the presence of biotin on the nascent DNA can interfere with its subsequent utilization in cloning or hybridization analysis."

Thus, applicants submit that modification proposed by the Office would render Uhlmann '99 unsatisfactory for its intended purpose (see MPEP §2143.01, V.)

As previously noted, one widely accepted indicator that a modification would not have been obvious is, that the modification proposed would render the reference, here Uhlmann '99, unsatisfactory for its intended purpose.

MPEP §2143.01, V. cites *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984): In *In re Gordon*, the claimed device was a blood filter. The prior art taught a liquid strainer for removing dirt and water from gasoline. The Board concluded the claims were *prima facie* obvious, reasoning that it would have been obvious to turn the reference device upside down to arrive at the claimed invention. The court reversed, finding that if the prior art device was turned upside down it would be inoperable for its intended purpose because, among others, the gasoline to be filtered would be trapped.

Here, rather than turning the device around (*In re Gordon*), the Office, while citing Uhlmann' 99 as teaching the amplification required by the presently claimed invention, suggests, in order to arrive at the claimed invention, to use Nyren's biotin labeled amplification primer in a way that would render Uhlmann '99 inoperable for its intended purpose, namely accurately determining changes in methylation patterns, here by two dimensional DNA fingerprinting.

The Office expressed the opinion that since the intended purpose of Uhlmann '99 is determining the methylation status of DNA, Uhlmann '99 would not be rendered inoperative by making the substitution that the Office suggested. The Office seems to argue that the intended purpose of Uhlmann is to arrive at the presently claimed invention. Applicants respectfully disagree.

The Office is in this context also directed to *In re Ratti*, 270 F.2d 810, 813 (CCPA 1959) were the court note that the "suggested combination of references would require a substantial reconstruction and redesign of the elements shown in [the primary reference] as well as a change in the *basic principles* under which the [primary reference] construction was designed to operate." (Emphasis added)). In *Ratti*, the modification suggested by the Office changed the basic principle of sealing from attaining sealing through a rigid, press-fit, interface between the components, to attaining sealing by providing a resilient interface between the components. *Id.* at 811-13. It was found that such a modification fundamentally changes the technical basis of how a seal performs its sealing function and how a sealed interface is attained.

Applicants respectfully submit that the Office's analysis change the *basic principles* under which Uhlmann '99 was designed to operate. Thus, the previously submitted evidence about biotylated primers for cloning is relevant for the instant case and should be considered. The Office is directed to applicants' argument as presented in the response of May 28, 2008.

In view of the reasons provided above, applicants respectfully submit that the Office's analysis does not support a *prima facie* case of obviousness.

Applicants submitted that the combination as suggested would render the reaction mixture more complex, leading to potential technical difficulties. The Office stated just because one reaction mixture is more complex than another reaction mixture does not mean that it does not work. However, the complexity of the reaction mixture goes to motivation of making the combination the Office suggested. Uhlmann '99 aims at improved detection (not speed). Uhlmann thus does not provide any motivation for the combination the Office made. Applicants submit that providing a more complex reaction mixture provides a disincentive for the proposed modification, especially against the backdrop that improvement in a method, such as improved detection, on the one hand, and speed, on the other hand, are often considered to be at conflict.

Applicants note again that an obviousness analysis starts out at the prior art, not at the claimed invention. The question is, whether it would have been obvious, at the time the invention was made, to combine and/or modify the prior art to arrive at the claimed invention.

In this context, applicants previously direct the Office's attention to a recent discussion of non-obviousness in *Ortho-McNeil Pharmaceutical v. Mylan Labs,* 2007-1223, Fed Cir. March 31, 2008. In particular, applicants directed the Office's attention to, Judge Radar notation that "In retrospect, Dr. Maryanoff's pathway to the invention, of course, seems to follow the logical steps to produce these properties, but at the time of invention, the inventor's insights, willingness to confront and overcome obstacles, and yes, even serendipity, cannot be discounted." (page 10). The Office stated that hindsight like reasoning is only improper if it included knowledge gleaned only from applicants disclosure. In this context, applicants note that in *Ortho-McNeil* the court specifically stated that the TSM test, flexibly applied (in the unpredictable arts) merely assures that the obviousness test proceeds on the basis of evidence – teachings, suggestions (a tellingly broad term), or motivations (an equally broad

term) – that arise before the time of invention as the statute requires. Applicants respectfully submit their belief that, for the reasons provided above, the appropriate showing was not provided.

With regard to claim 12, the Office explains on page 17 and 18 that part of the limitation (d), namely the "to diagnose a pathological condition" or the predisposition therefore is not an actual step, but an intended use of claim limitation (d):

> "(d) detecting whether said nucleotide is methylated or not methylated at said predetermined position in the sample to diagnose said pathological condition or the predisposition for said pathological condition."

While, the Office expressed the opinion that Uhlmann '99 teaches an association between hypomethylation and piocytic astromas, Nyren teaches that pyroseqeuncing can be used to detect disease and Herman teaches that the detection of methylated CpG is indicative of several disorders, the Office has not explained how, subsequent to the suggested modification of Uhlmann '99, the recited detection may take place. Clarification is respectfully requested.

With regard to claims 34 and 39, the Office concedes that Sylvan only an expectation that an allele frequency of 5% with a standard deviation of not more than 1% is detected, but suggests that, even if the expected results do not end up being equivalent to the [claimed] results, it would have been obvious to modify the method in order to determine the recited allele frequency.

The Office has not provided any indication how the reference is to be modified to accomplish this. In this context, the Office is in particular directed to Sylvan's Fig. 6 supporting the Office's remark that the expected results do not end up being equivalent to the [claimed] results. Clarification is respectfully requested.

Applicants have shown above that no *prima facie* case of obviousness was established for independent claims 1, 12 and 32. A separate argument was provided for claims 34 and 39. Reconsideration of the rejections in view of applicants' arguments presented herein is respectfully requested.

No fee is believed to be due. However, the Commissioner is authorized to charge or credit deposit account no. 50-3135 as required. Any petition that may be required for the consideration of this response is herewith respectfully requested.

Respectfully submitted,

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